# Copepod track data from a set of 4 experiments quantifying the foraging behavior and ingestion by copepods within different distributions of marine snow

**Website**: https://www.bco-dmo.org/dataset/880645
**Data Type**: experimental
**Version**: 1
**Version Date**: 2022-09-19

**Project**
» CAREER: Small-scale plankton-aggregate dynamics and the biological pump: Integrating mathematical biology in research and education (PlanktonAggDyn)



| Contributors | Affiliation | Role |
|---|---|---|
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**Abstract**
This dataset results from a set of four experiments conducted to quantify the foraging behavior and ingestion by copepods within different distributions of marine snow. Experiments were conducted at the University of San Diego in the Prairie research lab. This dataset includes Matlab files containing the copepod track data from these experiments.

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## Dataset Description

Below are the descriptions of the Matlab ".mat" data files in the attached file "MarineSnowLayers_CopepodTracksData.zip".

All Matlab files are named in the following format:

*EXPX_TR_CAMYY_Track_ZZ*

where "X" will be 1, 2, 3, or 4 corresponding to the Experiment number of the track,
"TR" will be CG (representing control with gradient treatment), CNG (representing control with no gradient treatment), Homogenous (representing homogenous treatment), or Layer (representing layer treatment),
"YY" will be either 01 or 02 representing the camera number, and
"ZZ" will be a number representing the copepod track number.

The total number of tracks varies by treatment and experiment according to the table below:

| Exp. | Treatment | Camera | Number of Tracks |
|---|---|---|---|
| 1 | CG | 01 | 36 |
| 1 | CNG | 01 | 28 |
| 1 | Homogenous | 01 | 25 |

| | | | |
|---|---|---|---|
| 1 | Layer | 01 | 52 |
| 1 | CG | 02 | 20 |
| 1 | CNG | 02 | 26 |
| 1 | Homogenous | 02 | 34 |
| 1 | Layer | 02 | 40 |
| 2 | CG | 01 | 37 |
| 2 | CNG | 01 | 23 |
| 2 | Homogenous | 01 | 42 |
| 2 | Layer | 01 | 69 |
| 2 | CG | 02 | 26 |
| 2 | CNG | 02 | 25 |
| 2 | Homogenous | 02 | 28 |
| 2 | Layer | 02 | 50 |
| 3 | CG | 01 | 26 |
| 3 | CNG | 01 | 23 |
| 3 | Homogenous | 01 | 23 |
| 3 | Layer | 01 | 62 |
| 3 | CG | 02 | 15 |
| 3 | CNG | 02 | 21 |
| 3 | Homogenous | 02 | 25 |
| 3 | Layer | 02 | 30 |
| 4 | CG | 01 | 27 |
| 4 | CNG | 01 | 30 |
| 4 | Homogenous | 01 | 19 |
| 4 | Layer | 01 | 59 |
| 4 | CG | 02 | 28 |
| 4 | CNG | 02 | 20 |
| 4 | Homogenous | 02 | 32 |
| 4 | Layer | 02 | 52 |

Each Matlab file contains the same three vectors depending on the camera:
*cop_xloc_cm* (for Camera 1) or *cop_yloc_cm* (for Camera 2)
*cop_zloc_cam1_cm* (for Camera 1) or *cop_zloc_cam2_cm* (for Camera 2)
*time_cam1* (for Camera 1) or *time_cam2* (for Camera 2)

The *cop_xloc_cm*/*cop_yloc_cm* provide the horizontal location of the copepod in cm from the edge of the tank (defined as the x direction for Camera 1 and the y direction for Camera 2).

The *cop_zloc_camX_cm* vectors provide the vertical location of the copepod in cm from the bottom of the tank in Cameras 1 and 2, respectively.

And the *time_camX* vectors provide the time in seconds since the first copepod appeared in the field of view.

All three of the vectors in each individual Matlab file are the same length and indicate the number of sequential images that the copepod was tracked.

**Methods & Sampling**

During the autumn of 2020, four experiments were conducted in which copepods of the species *Calanus pacificus* were exposed to four different feeding environments (treatments). These four treatments were: a tank with a marine snow layer, a tank with a homogenous distribution of marine snow, and two control treatments without food – one with a density gradient and one with no density gradient. Copepods were recorded with two cameras, allowing us to reconstruct 2D and 3D copepod tracks. From these tracks, we determined vertical distributions of copepods and quantified behavioral properties, including swimming velocity, a measure of path linearity, and jump frequency. We also measured copepod ingestion in the four treatments using gut pigment analysis.

*C. pacificus* was collected using a small boat near Scripps Canyon in La Jolla, CA (32° 51.720′ N, 117° 16.816′ W) 11-22 days before each experiment with a 333 µm mesh plankton net (0.5 m diameter mouth). Samples were sorted in the lab to isolate individuals of the species *C. pacificus*. Copepods were maintained with regular water changes in an incubator in the dark at 18°C until the experiment and fed *Thalassiosira weissflogii* . Twenty-four hours prior to each experiment, a total of 120 copepods were starved (30 copepods for each treatment). The copepods for each treatment were placed in separate 100 mL beakers filled with filtered seawater and stored in complete darkness at room temperature (~21 ˚C) until just prior to the experiment. For each treatment, the copepods were starved in filtered seawater with a density equal to that of the top layer fluid for that treatment, which differed between treatments because of how each feeding environment was created. This allowed for the copepods to be acclimated to the density of the top layer fluid in their assigned treatment tank to limit any shock or behavioral reaction at the beginning of the experiment.

Sixteen days prior to each experiment, four identical cultures of *T. weissflogii* were started with a concentration of 10,000 cells/mL in 1.8 L of f/2 media (two each for the layer treatment and the homogenous treatment). These cultures were kept on a 12:12 hour light:dark cycle at room temperature and phytoplankton concentration was measured every other day with a particle counter (Beckman Coulter). The phytoplankton cultures were grown for a total of thirteen days, corresponding to roughly the middle of their exponential growth phase.

After the phytoplankton cultures grew for thirteen days, and three days prior to the experiment, each *T. weissflogii* culture was diluted to 32,500 cells/mL and transferred into a cylindrical acrylic tank with a volume of 2.2 L and circumference of 51 cm. The cylindrical tanks used to form aggregates designated for the layer treatment were filled with seawater with density equal to the top layer fluid for that treatment. The tanks used to form aggregates designated for the homogenous treatment were filled with seawater that had a density ~0.0020 g/cm3 less (i.e. a salinity of ~1.3 psu less) than the fluid for that treatment; this was done to allow for slower aggregate settling speeds in that treatment, based on observations in preliminary experiments. The cylindrical tanks were placed on a roller table and were allowed to rotate at a speed of 4.3 RPM for 3 days in the dark to form aggregates.

A single experiment consisted of a set of four different treatments. These treatments were done one after the other, in the following order: control with gradient, layer, homogenous, and control with no gradient. The four treatments were all created in a rectangular acrylic tank of dimensions 10 cm x 10 cm x 50 cm. In each treatment, 25 copepods were allowed to feed in the tank for between 4-8 minutes.

The control with gradient treatment and the layer treatment required the formation of a density gradient. This density gradient allowed for the formation of an ephemeral marine snow layer (lasting about 8 minutes) in the layer treatment. An identical density gradient was created in the control with gradient treatment so we could account for any potential changes in copepod behavior in response to the change in density. Filtered seawater was used for the bottom layer fluid, which had a density between 1.0233-1.0236 g/cm3 (salinity between 33.6-34.0 psu) at room temperature, ~21 ˚C. Filtered seawater was diluted with DI water to a target density of 0.0040 g/cm3 less (or salinity of 5.3 psu less) than the bottom layer fluid to create the top layer fluid. To form the density gradient, we filled the tank with bottom layer fluid to ~25 cm from the bottom of the tank (approximate halfway mark). In order to get rid of any bubbles adhering to the sides of the tank, which might obstruct a clear camera view, we ran a sponge along the inside tank walls. Then, top layer fluid was carefully poured on top of the bottom layer fluid through a diffuser made from a sponge that had been soaked in top layer fluid. This diffuser floats at the surface of the water column and prevents mixing at the density interface as the less dense top layer fluid is added to the tank slowly with a pump (Micropump Model GB-P23.JVS.A.B1). The homogenous treatment and the control with no density gradient treatment did not require a density gradient, and instead were filled entirely with undiluted filtered seawater (i.e., bottom layer fluid from the other treatments).

Just prior to starting each treatment, the starved copepods that were set aside for each treatment were transferred from their 100 mL beakers into 10 mL beakers. This made it possible to pour the entire beaker into a ladle which was used to transfer the copepods into the tank. For treatments that required marine snow aggregates (the layer treatment and the homogenous treatment), the cylindrical tank containing aggregates was carefully taken off the roller table and placed upright so that the aggregates slowly settled to the bottom of the tank. The marine snow aggregates were then carefully transferred into a small glass vial, so as not to break up the fragile aggregates, such that the total volume of aggregates in the vial was equal to roughly 5 mL.

Once the tank was set up, the copepods and aggregates were added accordingly, based on the treatment. For both control treatments, the copepods were placed into the tank by transferring them with a ladle, which was gently placed at the surface of the water and tilted so the copepods were released into the tank. For the layer treatment, the aggregates were pipetted into the tank just below the surface, such that they had a relatively even horizontal distribution. The copepods were ladled into the tank once a distinct marine snow layer had formed. For the homogenous treatment, about half of the aggregates were pipetted just below the surface into the tank, again such that the distribution of aggregates horizontally was fairly homogenous. Then all 25 copepods were ladled into the tank, with the remaining aggregates added afterwards. This method created a roughly homogenous distribution of aggregates sinking around the copepods throughout the time of camera recording.

The experimental tank was set up on a table and was lit from below with a near-infrared light-emitting diode (LED) aimed upwards through a Fresnel lens through a cut-out in the table with an overlying piece of clear plexiglass. Two near-infrared sensitive cameras (Point Grey Grasshopper Camera Model GS3-U3-41C6NIR-C) were set up at 90˚ angles facing two neighboring sides of the tank. During the experiments, the cameras recorded at 12 frames per second. The recording started immediately prior to adding the marine snow aggregates to the tanks (or the copepods in the case of the control treatments). For the two control treatments, the recording was stopped once the copepods had been in the tank for approximately 8 minutes. For the layer treatment, the recording was stopped once the aggregates started falling out of the layer. For the homogenous treatment, the recording was stopped once the aggregates started sinking out of the bottom of the field of view to limit the amount of time that the animals may be feeding on aggregates sitting on the bottom of the tank. The images had a field of view of ~30 cm x 10 cm. This field of view is vertically centered, so that it is ~10 cm from the bottom of the tank and ~10 cm from the top of the tank. The density gradient, which is located ~25 cm from the bottom of the tank, is located at roughly the halfway mark in the field of view (~15 cm from both the bottom and top of field of view).

Once copepod behavior had been recorded, the cameras were turned off and the copepods were carefully siphoned out of the tank onto a 100 µm mesh sieve. All the water from the tank was saved in a bucket for filtering after the experiments. Copepods were then collected for gut pigment analysis. After the copepods were removed from each treatment tank, using both cameras we recorded images of a ruler aligned vertically in the center of the tank, which were used for image calibration.

After the copepods were siphoned out of the tank and onto the sieve, pairs of copepods were added to amber vials filled with 3mL of 90% acetone (10 per treatment), recovering 20 of the copepods in each treatment. Copepods in each vial were sonicated at 40% amplitude for 5 seconds to break up the organisms and release gut content into the acetone. The amber vials were then placed in a -20˚C freezer overnight. The water of each treatment tank was also filtered and analyzed for chlorophyll concentration. Prior to filtering, the water from each tank was well mixed, and then three subsamples of 25 mL each were filtered onto a GF/F filter. These filters were then placed in amber vials filled with 5 mL of 90% acetone to extract the chlorophyll into solution and the vials were placed into a -20˚C freezer overnight. The following day, the copepod gut and tank water samples were analyzed using a fluorometer (Trilogy, Turner Designs) to measure the concentration of chlorophyll and pheophytin in the acetone solution.

This data set includes the copepod track data from these experiments. For the copepod gut pigment data from the same experiments, see related dataset https://www.bco-dmo.org/dataset/880669.

**Data Processing Description**

**Data Processing:**
All images in every treatment were processed in MATLAB. The images were cropped horizontally to remove the tank walls on the left- and right-hand sides of the image which can interfere with tracking copepods. Once the images were cropped, an image from before any copepods or aggregates were added to the tank was subtracted from all other images to remove constant background parts of the images (e.g., tank walls, air bubbles, etc.).

Since the copepods and aggregates are light against a dark background, we applied a threshold to the images with which we could identify copepods and aggregates; a group of connected pixels over a defined threshold were identified as a potential copepod or aggregate. Copepods were manually identified by eye when they first entered the field of view, and their x- and y-coordinates in the first image they appeared were used to initiate the tracking of each individual.

Copepod tracks were then created for each camera separately by matching pixels identified as copepods in subsequent images. Copepods were matched by minimizing the change in distance between individual copepods in neighboring frames, and accounting for a maximum distance that can be traveled between frames. In some cases, copepod tracks were not able to be constructed automatically through this process because of the close proximity to other copepods or aggregates. In these cases, copepods were either manually tracked for a period of time until they could be automatically tracked again, or that track was ended and a new track was created if and when the copepod reappeared. Copepods that exited through the bottom or top of the field of view or that would swim too near to the wall would also result in those tracks being terminated.

The copepod track coordinates were linearly converted from pixels to centimeters using the image of a ruler taken immediately following each treatment, defining copepod vertical location as distance (in cm) from the bottom of the tank.

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## Data Files

| File | |
|---|---|
| **MarineSnowLayers_CopepodTracksData.zip** | (ZIP Archive (ZIP), 14.54 MB)<br>MD5:78e37b55ec7a3406c789ab1a041ca20e |

Below are the descriptions of the Matlab ".mat" data files in the MarineSnowLayers_CopepodTracksData Folder/

All Matlab files are named in the following format:

EXPX_TR_CAMYY_Track_ZZ

where "X" will be 1, 2, 3, or 4 corresponding to the Experiment number of the track,

"TR" will be CG (representing control with gradient treatment), CNG (representing control with no gradient treatment), Homogenous (representing homogenous treatment), or Layer (representing layer treatment),

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"ZZ" will be a number representing the copepod track number.

Each Matlab file contains the same three vectors depending on the camera:

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cop_zloc_cam1_cm (for Camera 1) or cop_zloc_cam2_cm (for Camera 2)

time_cam1 (for Camera 1) or time_cam2 (for Camera 2)

The cop_xloc_cm/cop_yloc_cm provide the horizontal location of the copepod in cm from the edge of the tank (defined as the x direction for Camera 1 and the y direction for Camera 2).

The cop_zloc_camX_cm vectors provide the vertical location of the copepod in cm from the bottom of the tank in Cameras 1 and 2, respectively.

And the time_camX vectors provide the time in seconds since the first copepod appeared in the field of view.

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## Related Datasets

### IsRelatedTo

Prairie, J. (2023) **Copepod gut pigment and tank water pigment data from a set of 4 experiments quantifying the foraging behavior and ingestion by copepods within different distributions of marine snow.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-09-20 doi:10.26008/1912/bco-dmo.880669.1 [view at BCO-DMO]

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## Parameters

*Parameters for this dataset have not yet been identified*

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## Instruments

| | |
|---|---|
| **Dataset-specific Instrument Name** | near-infrared sensitive cameras (Point Grey Grasshopper Camera Model GS3-U3-41C6NIR-C) |
| **Generic Instrument Name** | Camera |
| **Generic Instrument Description** | All types of photographic equipment including stills, video, film and digital systems. |

| | |
|---|---|
| **Dataset-specific Instrument Name** | Beckman Coulter particle counter |
| **Generic Instrument Name** | Coulter Counter |
| **Generic Instrument Description** | An apparatus for counting and sizing particles suspended in electrolytes. It is used for cells, bacteria, prokaryotic cells and virus particles. A typical Coulter counter has one or more microchannels that separate two chambers containing electrolyte solutions. from https://en.wikipedia.org/wiki/Coulter_counter |

| | |
|---|---|
| **Dataset-specific Instrument Name** | incubator |
| **Generic Instrument Name** | In-situ incubator |
| **Generic Instrument Description** | A device on a ship or in the laboratory that holds water samples under controlled conditions of temperature and possibly illumination. |

| | |
|---|---|
| **Dataset-specific Instrument Name** | near-infrared light-emitting diode (LED) |
| **Generic Instrument Name** | LED light |
| **Generic Instrument Description** | A light-emitting diode (LED) is a semiconductor light source that emits light when current flows through it. Electrons in the semiconductor recombine with electron holes, releasing energy in the form of photons. |

| | |
|---|---|
| **Dataset-specific Instrument Name** | 333 micrometer mesh plankton net |
| **Generic Instrument Name** | Plankton Net |
| **Generic Instrument Description** | A Plankton Net is a generic term for a sampling net that is used to collect plankton. It is used only when detailed instrument documentation is not available. |

| | |
|---|---|
| **Dataset-specific Instrument Name** | Micropump Model GB-P23.JVS.A.B1 |
| **Generic Instrument Name** | Pump |
| **Generic Instrument Description** | A pump is a device that moves fluids (liquids or gases), or sometimes slurries, by mechanical action. Pumps can be classified into three major groups according to the method they use to move the fluid: direct lift, displacement, and gravity pumps |

| | |
|---|---|
| **Dataset-specific Instrument Name** | fluorometer (Trilogy, Turner Designs) |
| **Generic Instrument Name** | Turner Designs Trilogy fluorometer |
| **Generic Instrument Description** | The Trilogy Laboratory Fluorometer is a compact laboratory instrument for making fluorescence, absorbance, and turbidity measurements using the appropriate snap-in application module. Fluorescence modules are available for discrete sample measurements of various fluorescent materials including chlorophyll (in vivo and extracted), rhodamine, fluorescein, cyanobacteria pigments, ammonium, CDOM, optical brighteners, and other fluorescent compounds. |

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## Project Information

**CAREER: Small-scale plankton-aggregate dynamics and the biological pump: Integrating mathematical biology in research and education (PlanktonAggDyn)**

*NSF Award Abstract*:
The global carbon cycle is in part modified by marine biological processes, which can impact the amount of carbon that is transported from surface waters to the deep ocean. This project will investigate interactions between planktonic grazers and marine aggregates - sinking particles that form in the surface ocean and have been shown to play an important role in marine food webs. The small scale of these biological processes makes them particularly challenging to study, but modern advances in mathematics and computer science have made direct observations of these interactions feasible. Experiments using high-resolution imaging will provide direct visual observations of zooplankton ingestion and the alteration of marine aggregates. These laboratory studies will guide the development of mathematical models to examine how these interactions affect particulate carbon sinking out of the surface ocean. This project will support an educational initiative focused on training undergraduate biology students in mathematical and computational techniques. This initiative includes the development of new interdisciplinary courses and undergraduate-focused independent research projects to help prepare the next generation of scientists in quantitative techniques that are essential to tackling the most challenging and complex biological problems.

Marine snow aggregates are particles that form in the surface ocean from organic and inorganic matter. These aggregates play a fundamental role in the biological pump, as sinking particles are a dominant contributor to the downward transfer of carbon in the ocean. However, much of the small-scale processes governing these particles and their role in the marine carbon cycle are still unknown. The goal of this project is to use mathematical and computational techniques to investigate interactions between aggregates and planktonic grazers, an understudied link in the planktonic food web that has important implications for carbon export. Three-dimensional trajectories of copepods within marine snow thin layers will be obtained to experimentally investigate copepod foraging behavior in response to patchy distributions of marine snow. In addition, high-speed imaging will allow for the direct observation of how copepods manipulate and ingest marine snow aggregates, thus affecting their size and settling velocity. Lastly, a mathematical model will be developed to study the impact of these small-scale interactions on large-scale carbon cycling and export. This project will also support the implementation of a comprehensive education plan focused on teaching undergraduate students how mathematical modeling and computational techniques can be used to address biological questions. This educational objective will be accomplished through the development of new courses in mathematical and computational biology and through the inclusion of undergraduate students in independent research projects.

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## Funding

| Funding Source | Award |
| --- | --- |
| NSF Division of Ocean Sciences (NSF OCE) | OCE-1654276 |

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